



Towards an integrated approach for monitoring the effects of chemical contaminants in the Spanish coastal Mediterranean waters

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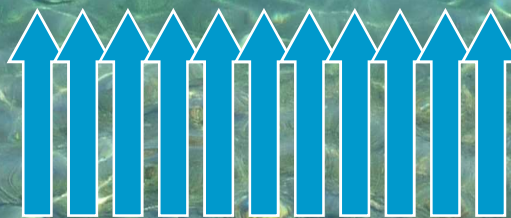
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Ecosystem-based approach



Marine health status



European Union
MSFD (2008/56/EC)

Ecosystem-based approach



Marine health status



European Union
MSFD (2008/56/EC)

Descriptor 8: Concentration of contaminants
are at level not giving rise to pollution effects

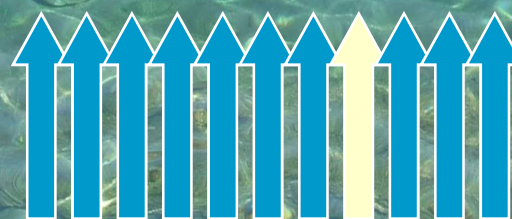
Ecosystem-based approach



Marine health status

ICES/OSPAR

MED POL



European Union
MSFD (2008/56/EC)

Chemical and biological effect
measurements

Water

Sediment

Biota

**OSPAR
Convention
CEMP**



**European
Union
MFSD**

**Barcelona
Convention
MED POL**

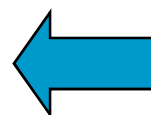




BARCELONA CONVENTION

**Environmental assessment
component**

**MED POL
PROGRAMME**



**Mediterranean
Action Plan**

MED POL Phase IV (2006-2013)

Strategy for the development of Mediterranean Marine
Pollution Indicators (MPIs)(UNEP, 2003)



MARINE ECOSYSTEM APPROACH: MPis

BIOLOGICAL INDICATORS

Number of exotic species (all taxa)
Number of zoobenthic species (S)
Presence/abundance of sensitive/opportunistic zoobenthic species/taxa
Community diversity (zoobenthos/phytobenthos)
Presence and coverage of benthic macrophytes (sensitive/opportunistic):POMI
Biotic index (EEI, BENTIX)

BIOMARKERS

Stress on stress (survival in air) in molluscs
Lysosomal membrane stability in molluscs and fish cells
Lipofuscin lysosomal accumulation in molluscs and fish cells
Neutral lipid lysosomal accumulation in molluscs and fish cells
Frequency of micronuclei in molluscs and fish cells
DNA damage in molluscs and fish cells
Peroxisome proliferation
Methallothionein in molluscs cells
AChE in molluscs
EROD activity

CHEMICAL INDICATORS

Referred to hazardous substances

HMB = heavy metals in Biota
HME= Heavy metals in Effluent
HMS = Heavy metals in sediment
OCB = Organochlorines in Biota
OCE = Organochlorines in Effluent
OCS = Organochlorines in Sediment
PHS = Petroleum Hydrocarbons in Sediment
PHB = Petroleum hydrocarbons in Biota
PHE = Petroleum hydrocarbons in Effluent
BBW = Bacteria in bathing waters
BSW = Bacteria in shellfish growing waters

Referred to eutrophication

Chl = Chlorophyll a
NO₃, NO₂, NH₄ = Nitrates, Nitrites and Ammonium
PO₄ = Orthophosphates
N, P = Total N and Total P
Tr = Transparency
BOD = Biological Oxygen Demand

Referred to the marine environment related to Climate Change

T/S = Temperature and Salinity

MEDPOL Phase IV (2006-2013):

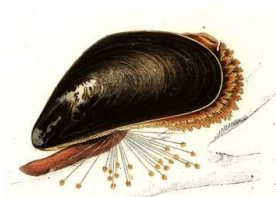
Strategy for the
development of
Mediterranean Marine
Pollution Indicators
(MPis), (UNEP, 2003)

Biomonitoring programs conducted by MCBE (IEO) along the Mediterranean coast: Main goals



BMCW program: Biomonitoring Program of contamination in Spanish Mediterranean coastal waters

- To establish the **spatial distribution** of selected contaminants and biological responses in native mussel populations in a number of coastal stations, hot spots and reference stations;
- To establish the **temporal trends** of selected contaminants and biological responses in native mussel populations in a number of coastal stations, hot spots and reference stations;
- To perform an **integrated assessment** of chemical quality of the coastal waters by using mussels and to monitor it over time.



**Contaminant
concentrations**

+

Biomarkers

Bionmonitoring program conducted by MCBE (IEO) along the Mediterranean coast: Main goals



BMIS program: Bionmonitoring Program of contamination in Spanish Mediterranean inner shelf ecosystem

- 1) To establish the **spatial distribution** and **temporal trends** of selected contaminants in superficial sediments in the target MED POL fish species red mullet (*Mullus barbatus*) (UNEP/RAMOGÉ, 1999) along the Iberian Mediterranean inner shelf;
- 2) To establish the **spatial variations** and **temporal trends** of biomarker responses in red mullet;
- 3) To establish the **toxicity associated** to the superficial sediments in areas along the Iberian Mediterranean inner shelf and assess them over time;
- 4) To perform an **integrated assessment** of chemical quality of the inner shelf bottoms and to monitor it over time.



Contaminant concentrations



Biological effects

CHEMICAL CONTAMINANTS



Recommendations of MEDPOL Programme and also considering OSPAR.

Use of reference materials, **QUASIMEME** and **IAEA** intercomparison exercises



TRACE METALS	Mercury
	Lead
	Cadmium
	Copper
	Zinc
	Arsenic
	Nickel
	Aluminium
ORGANOCHLORINATED COMPOUNDS	Polychlorinated byphenyls: CB28, CB52, CB101, CB105, CB118, CB138, CB153, CB156, B180.
	<i>pp'</i> DDE, <i>pp'</i> DDT y <i>op'</i> DDT
	γ -Hexachlorocyclohexane
	α -Hexachlorocyclohexane
	Hexachlorobenzene
	Transnonachlor
	Dieldrin
	Aldrin
POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)	Phenanthrene
	Anthracene
	Fluoranthene
	Pyrene
	Benzo[a]anthracene
	Chrysene
	Benzo[e]pyrene
	Benzo[b]fluoranthene
	Benzo[k]fluoranthene
	Benzo[a]pyrene
	Benzo[g,h,i]perylene
	Dibenzo[a,h]anthracene
	Indeno[1,2,3-c,d]pyrene

AAS: Graphite furnace, flameless atomic.

Cold vapour technique

GC: ECD detector, helium carrier gas

HPLC: UV detector, water-metanol gradient phase

BIOMARKERS and BIOASSAYS



BEQUALM and **MEDPOL** (Prof. A. Viarengo)



Mussel

Stress on Stress

Stability lysosomal

Micronuclei

AChE

Metallothioneins

} Two-tier
approach



Red mullet

Genotoxic damages

EROD

Micronuclei

AChE

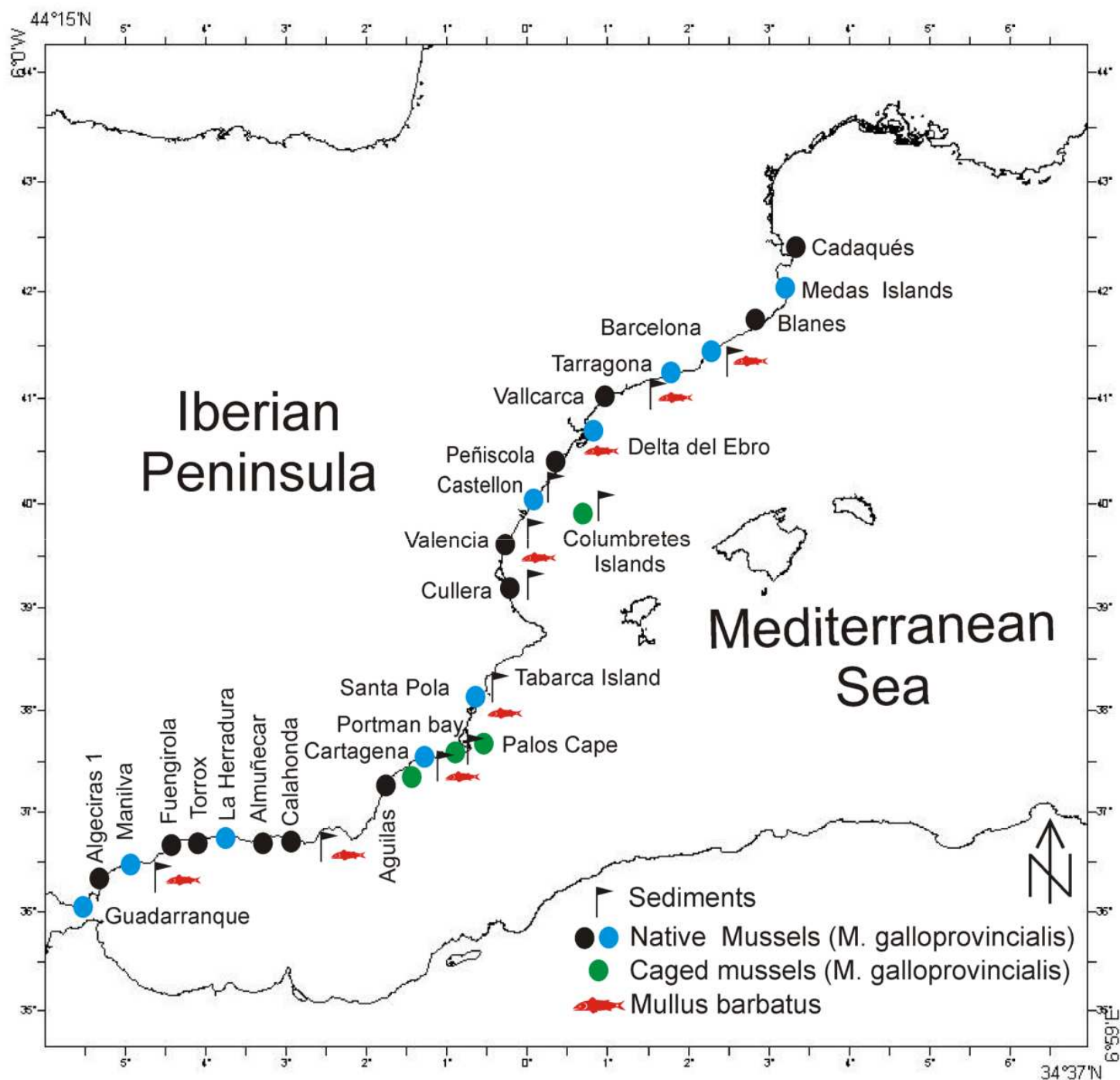
Metallothioneins

ALA-D



Sediments

Embryotoxicity test



BMIS program: Integrated sampling strategy

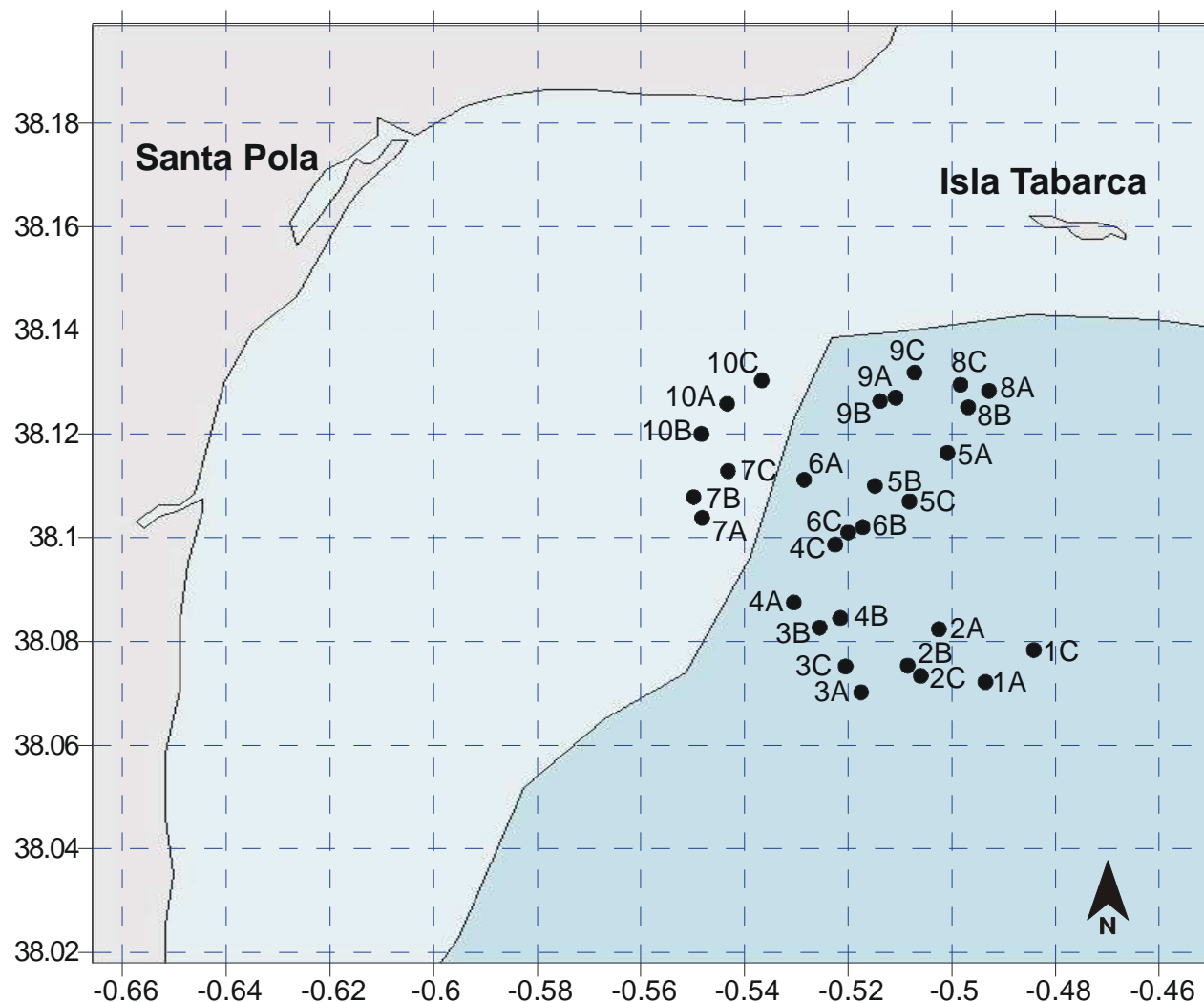


Sampling sediments: Pilot study 2006-2007-2008



SIZE SAMPLING: 3 samples/nautical mile²

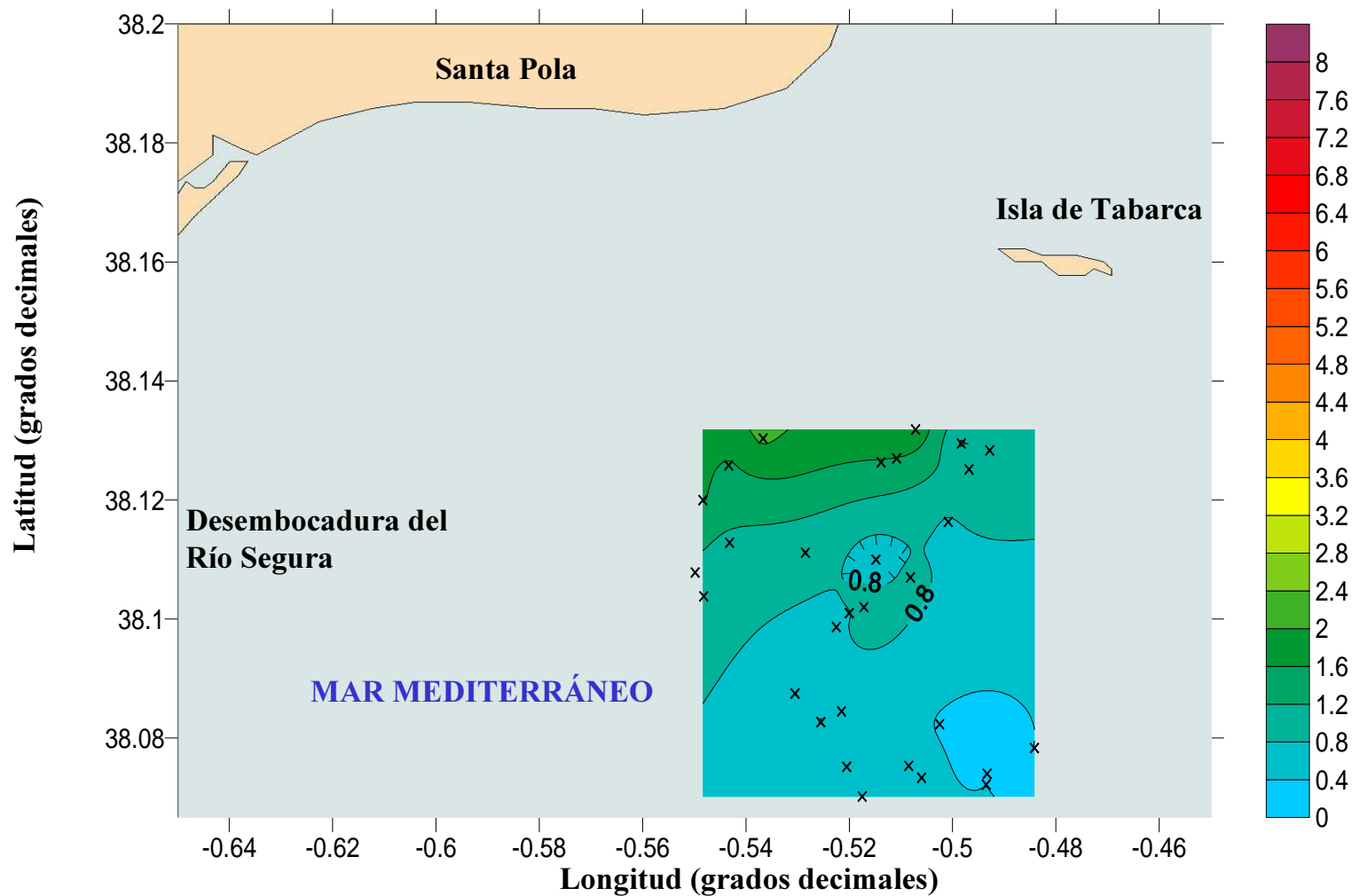
Analysis of contaminants in <2 mm fraction



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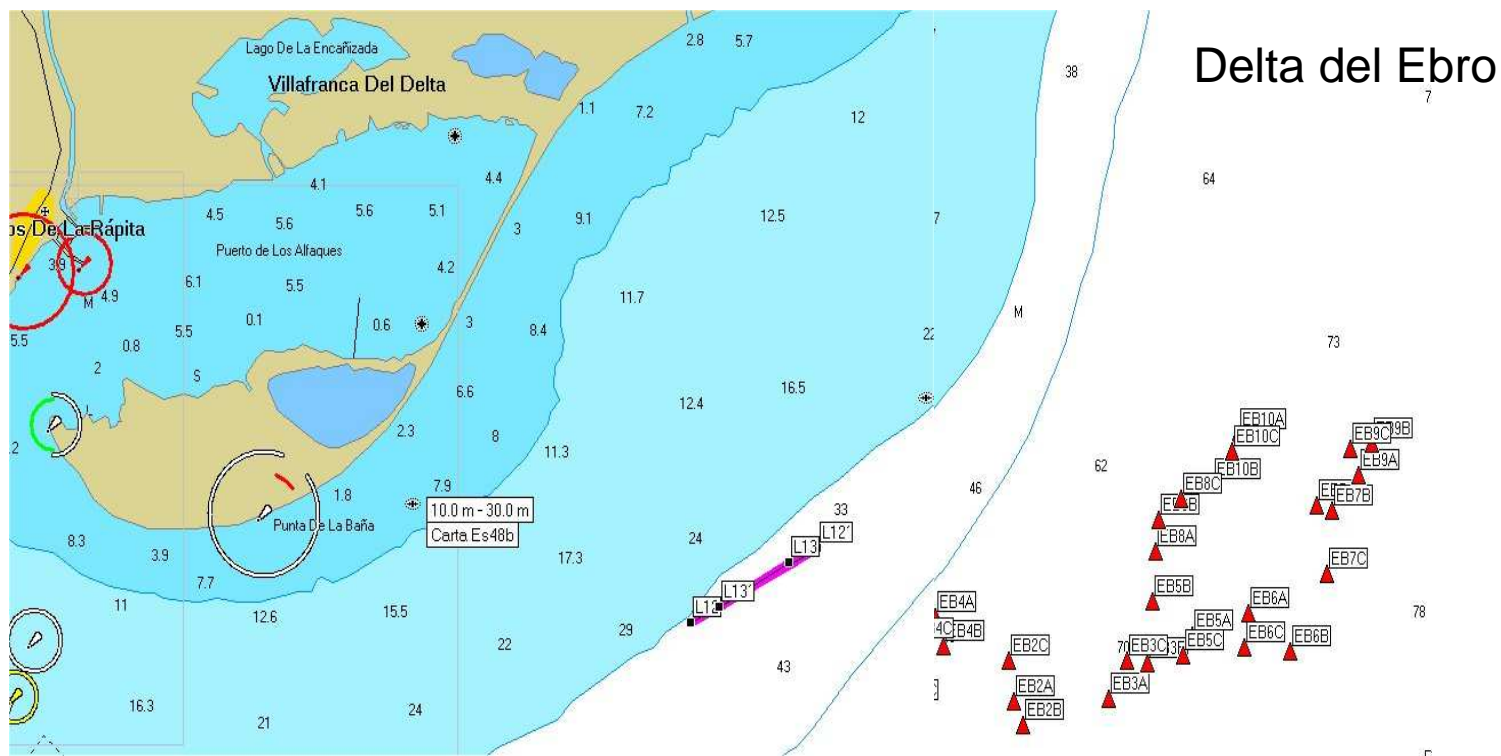


SIZE SAMPLING: 3 samples/nautical mile²
Analysis of contaminants in <2 mm fraction



BMIS program: Integrated sampling strategy

(fish and sediment components)

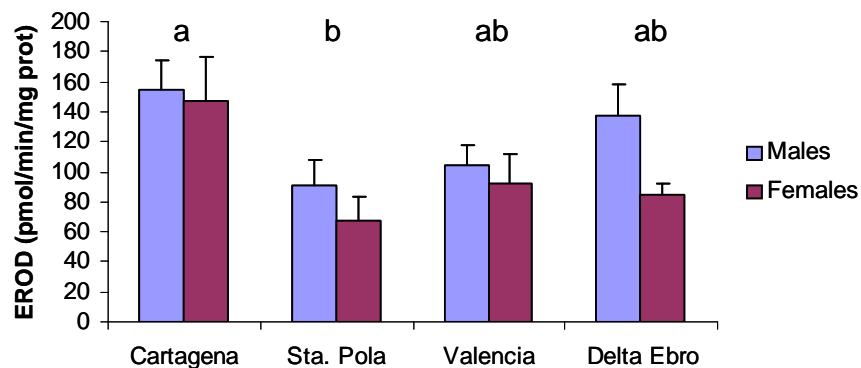


Some Preliminary Results

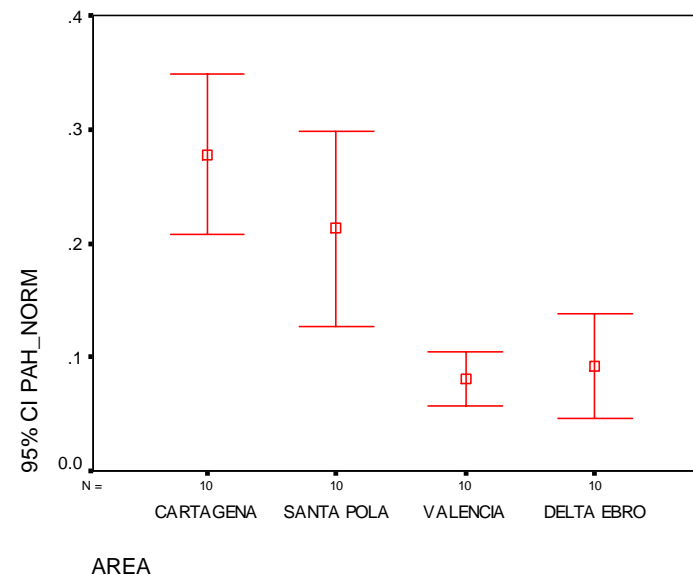
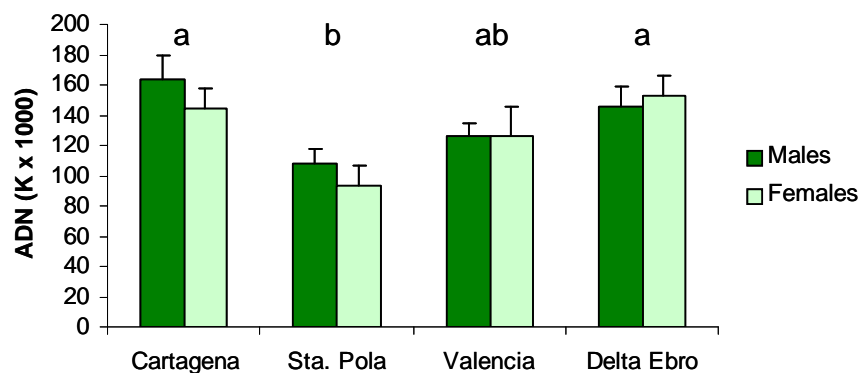
EROD activity , Genotoxic damages and PAH concentration in red mullet, 2006



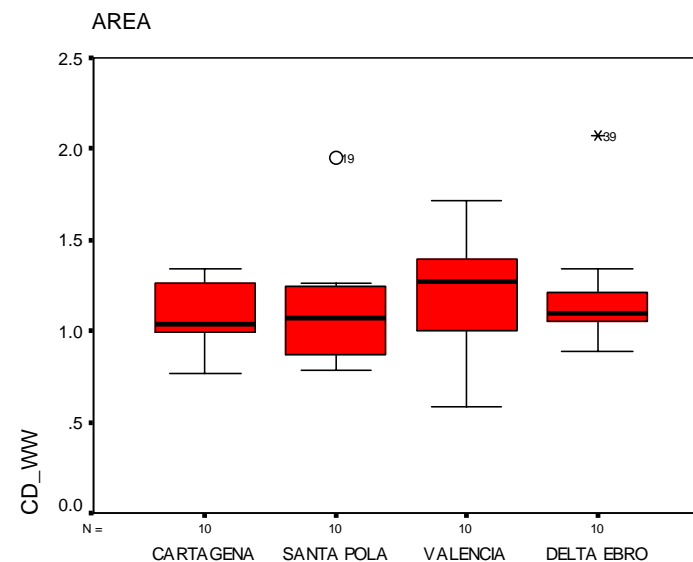
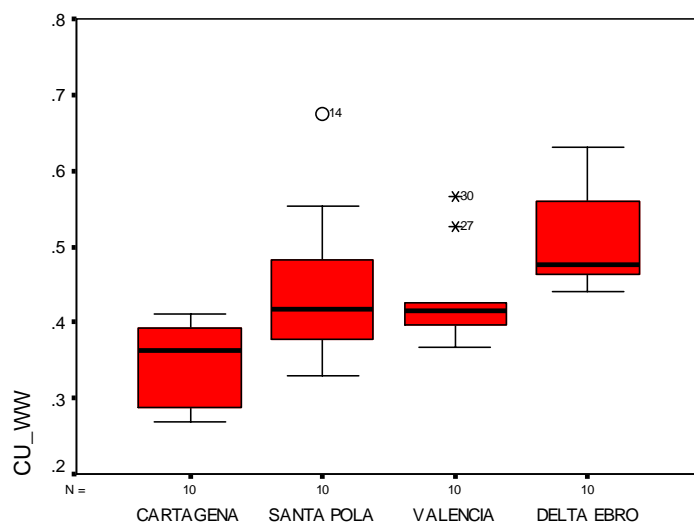
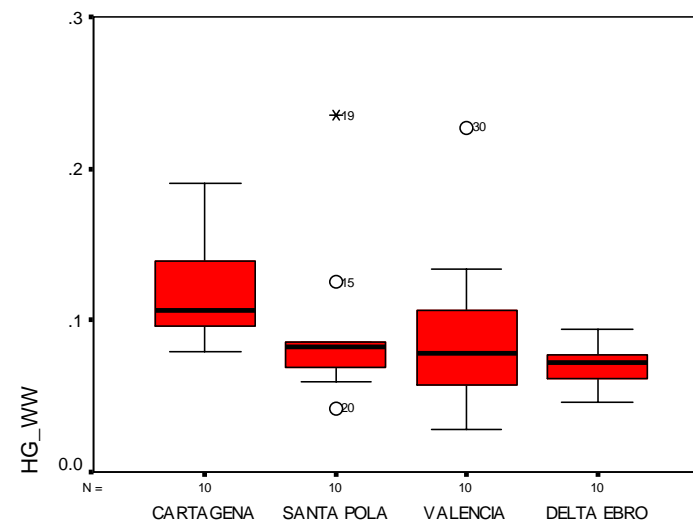
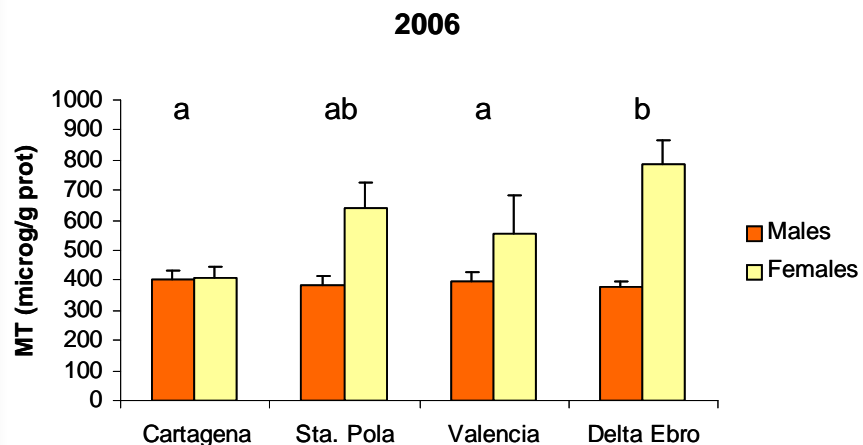
2006



2006



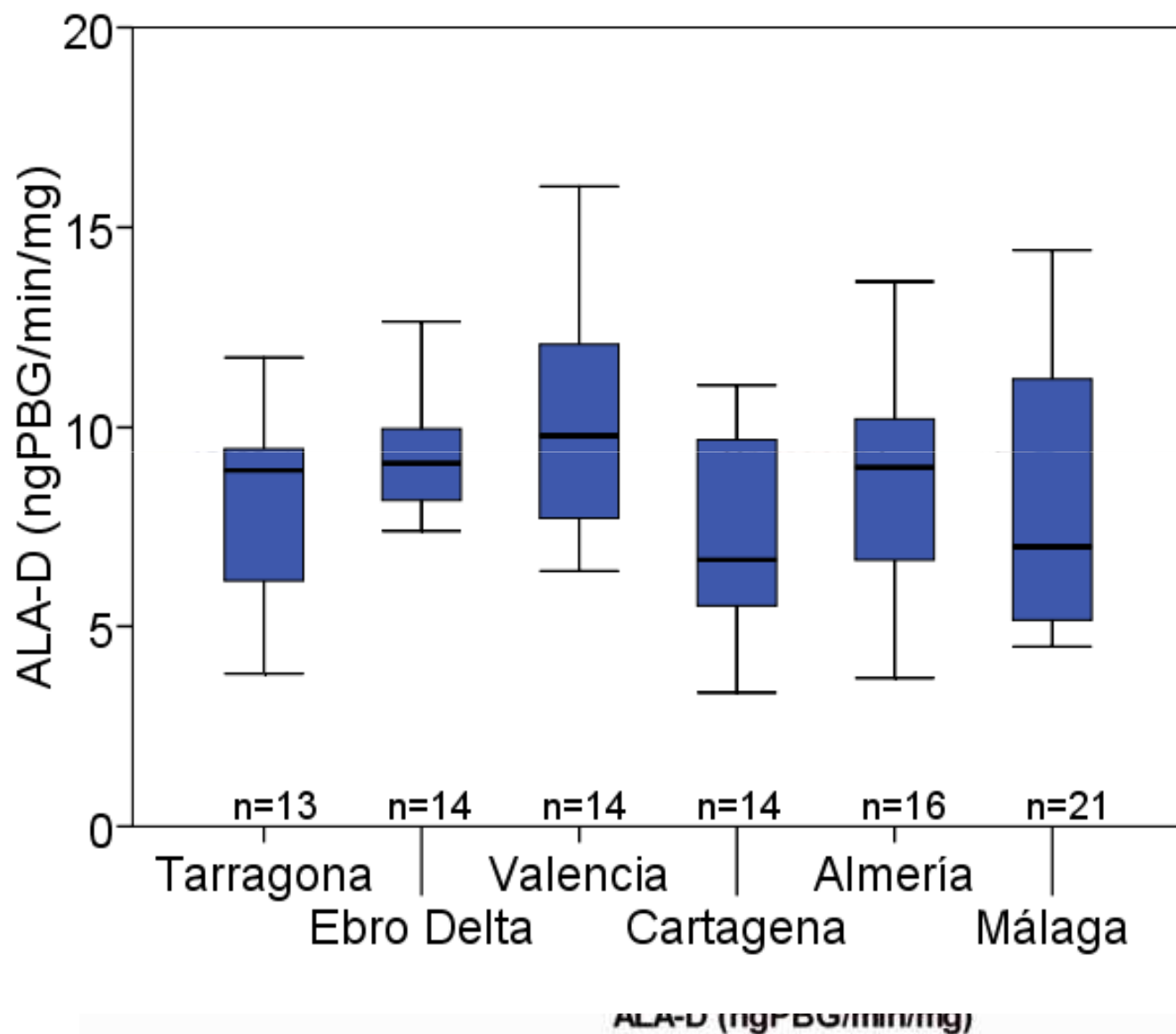
Metallothionein content, Hg, Cu and Cd concentration in red mullet, 2006.



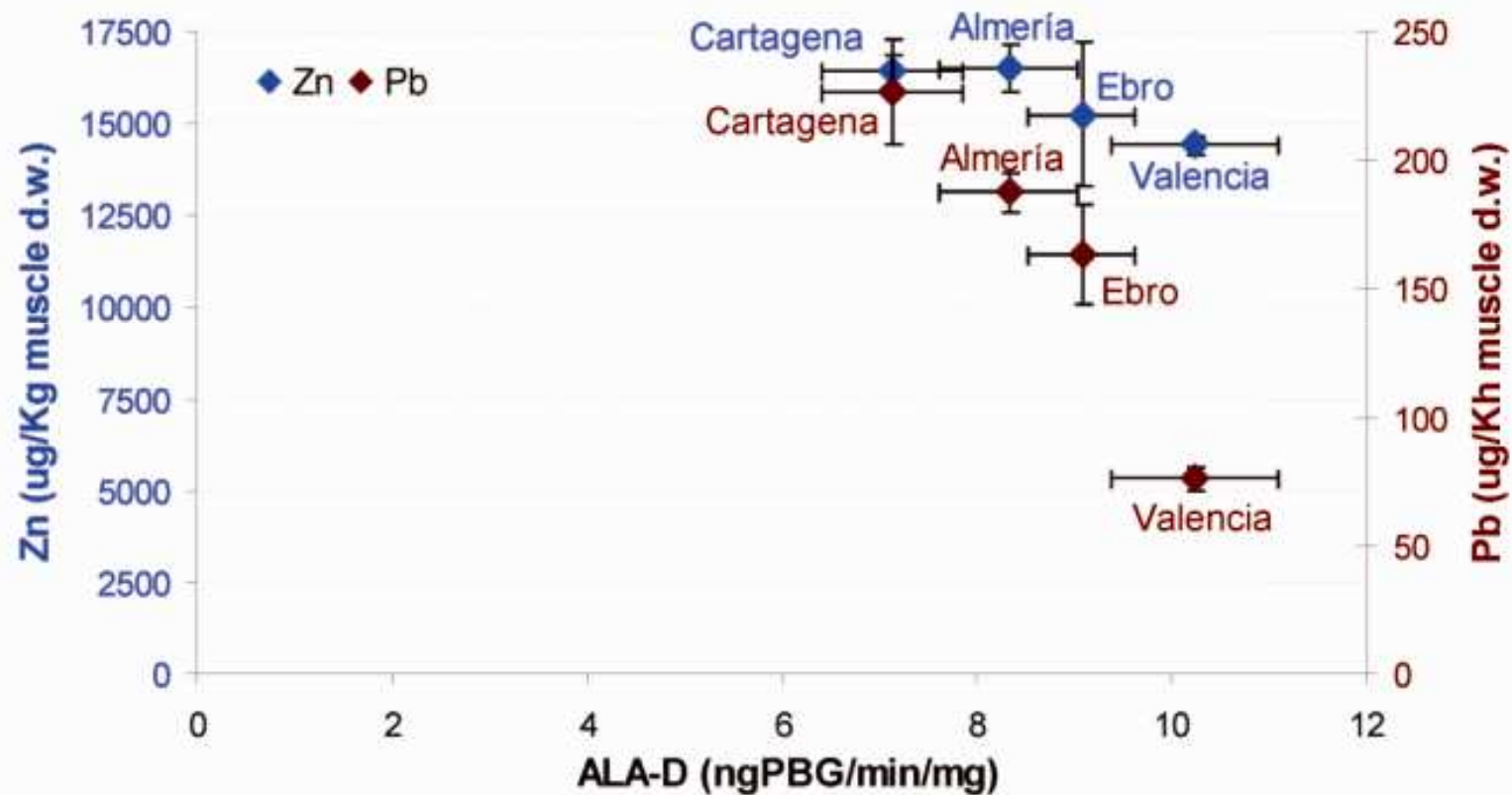
AREA

AREA

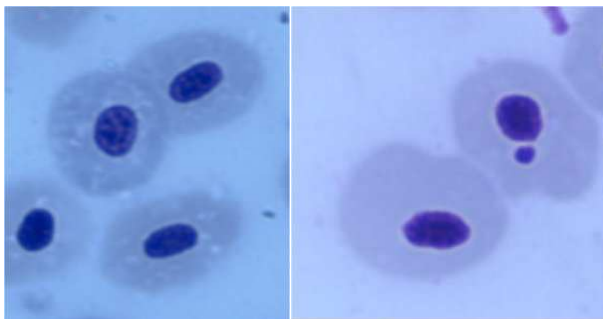
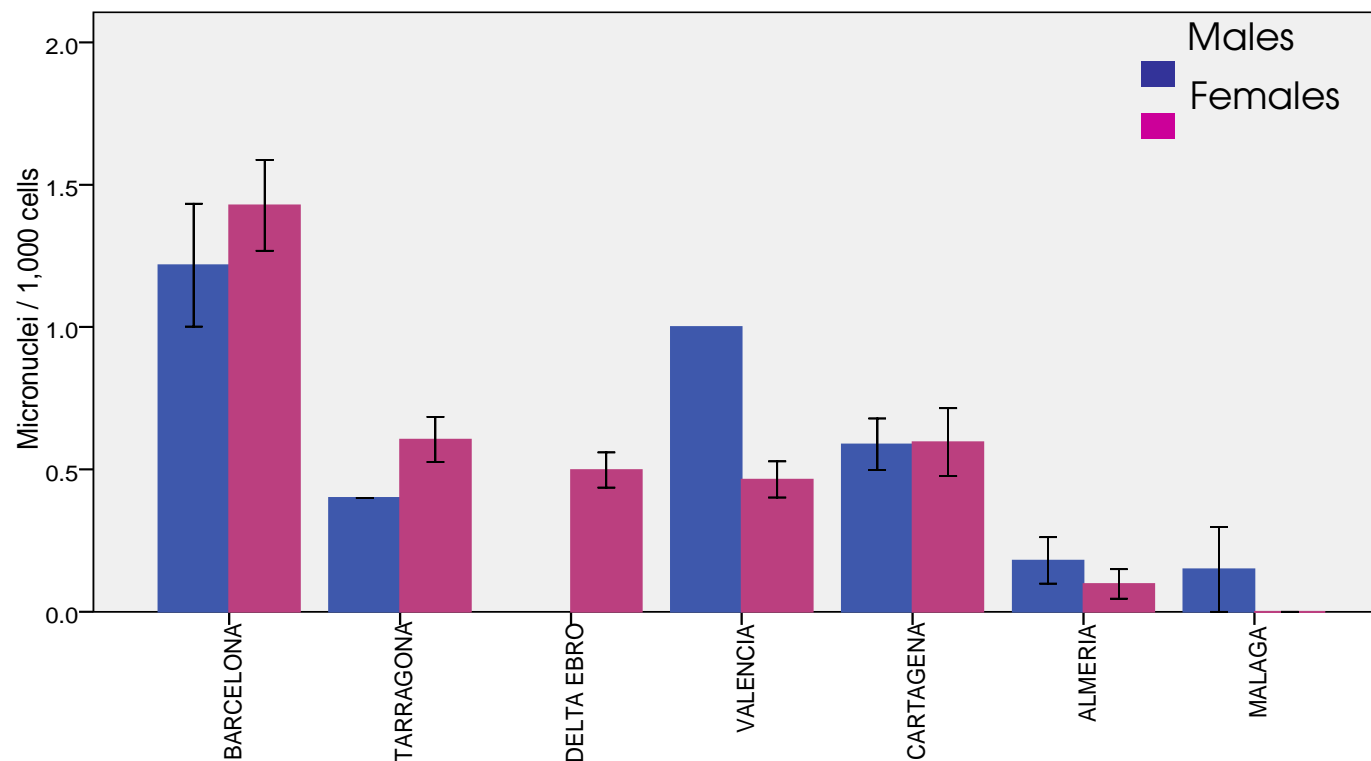
Ala-D activity in red mullet, 2008.



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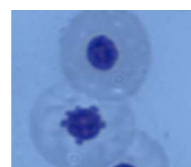
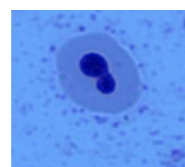
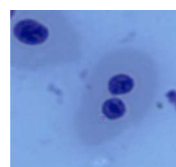
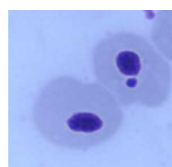
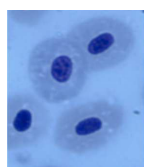
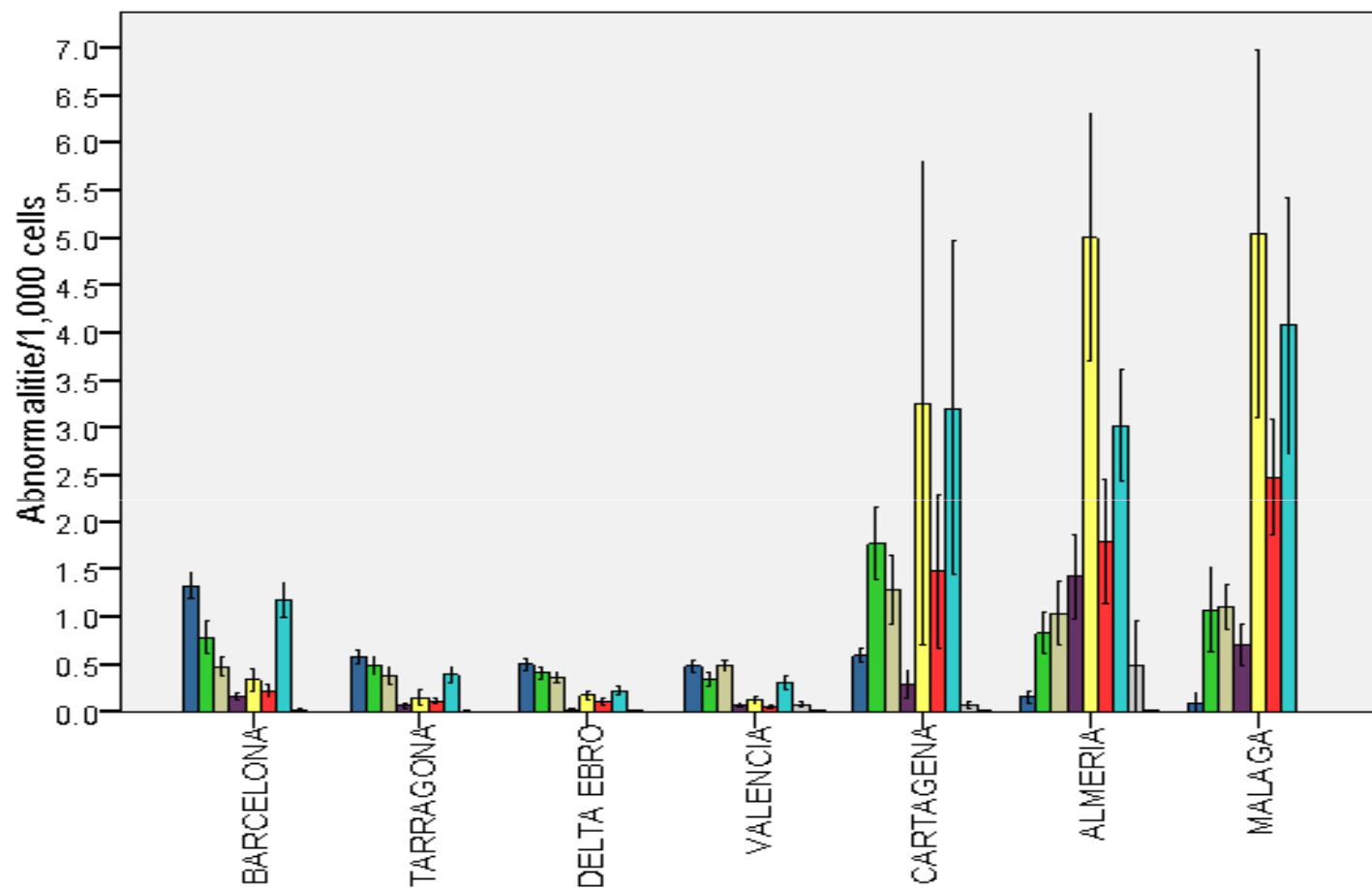


Nuclear abnormalities in red mullet, 2008.

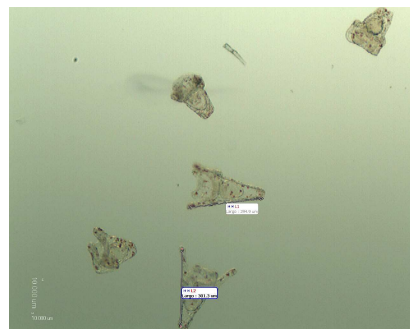
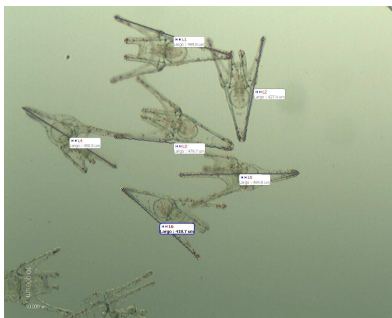


Highest MN frequency in erythrocytes of fish from Barcelona (1.3 ‰)

Nuclear abnormalities in red mullet, 2008.

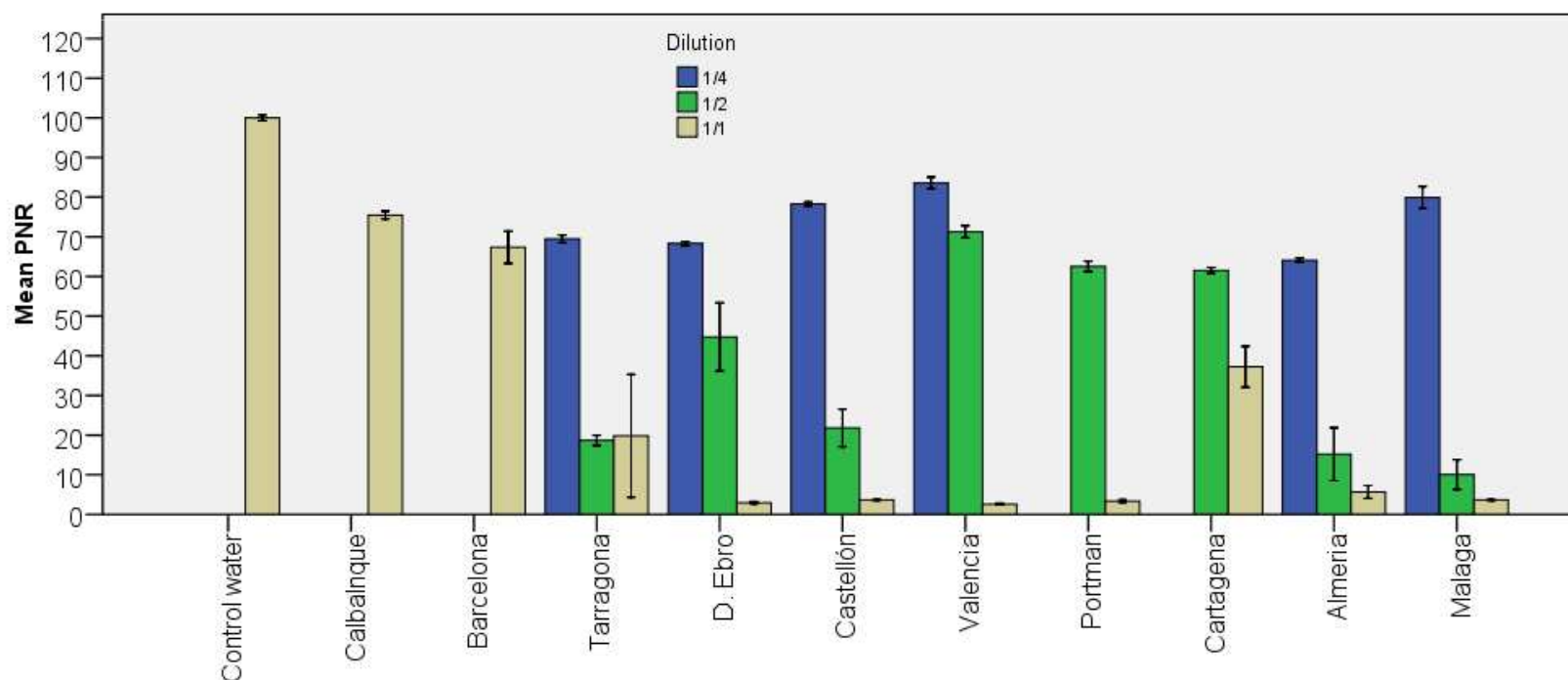


Contaminant in sediments, 2007-2008



PNR (Percentage Net Response)

$$\text{PNR} = (S1-S0) / (SC-S0)$$



Integrated assessment of chemical and biological effects measurements



- Develop ACs for MPIs
- Assess each of them in synthetic categories

Background
response

Elevated background
response

High and cause
for concern
response

- According to their ecological relevance, response of each MPIs will be weighted

Establishment of ACs for Biological responses in BMIS program



Background responses from presumed relatively unpolluted “reference” areas:

Valencia, Santa Pola, Almería and Málaga.

EROD in Red Mullet: ≤ 208 pmol/min/mg protein S9 in males
12-18 cm pre-spawning

Metallothionein: ≤ 571 mg/g in males 12-18 cm in males
pre-spawning

DNA integrity: $k \leq 0.172$ pooled sex 12-18 cm

Elevated biological response: Above the empirical 90 quantile

Establishment of ACs for Biological responses in BMIS program



Background levels from presumed relatively unpolluted “reference” areas:

Valencia, Santa Pola, Almería and Málaga.

MN frequency: Lowest found in fish from Almería (0.16 ‰) and Málaga (0.10 ‰).

Bolognesi et al., 2006 = 0.33 ‰ $\leq 0.33 \text{ ‰}$

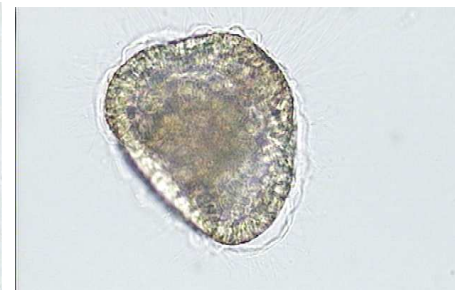
ALA-D background response (ng PBG min⁻¹ mg⁻¹)

- Red mullet 11-15
- Flounder and plaice: 13–21
- Atlantic cod 15-21
- dab 10-20

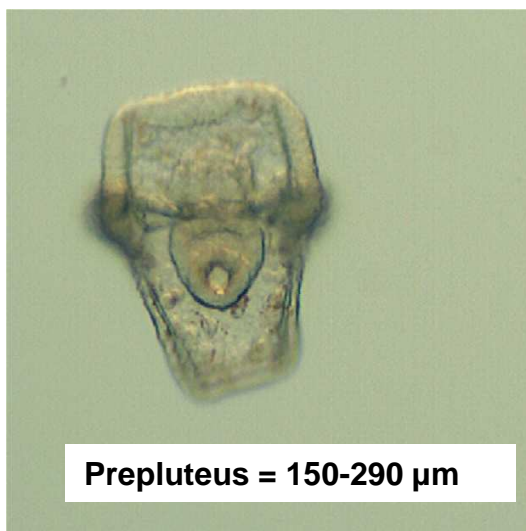
Assessment criteria to classify sampling sites into three different categories of sediment quality status (R. Beiras et al., 2010. ICES TIMES SERIES draft,)



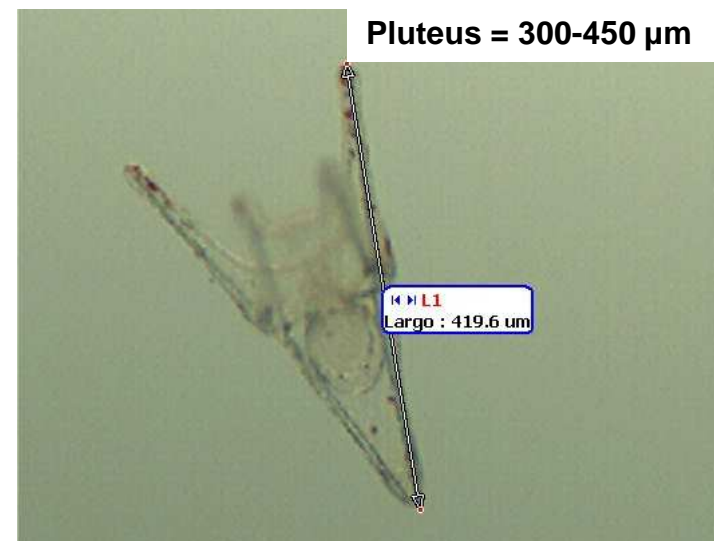
Fertilize eggs = $92 \pm 3 \mu\text{m}$



Gastrula eggs = 110-130 μm



Prepluteus = 150-290 μm

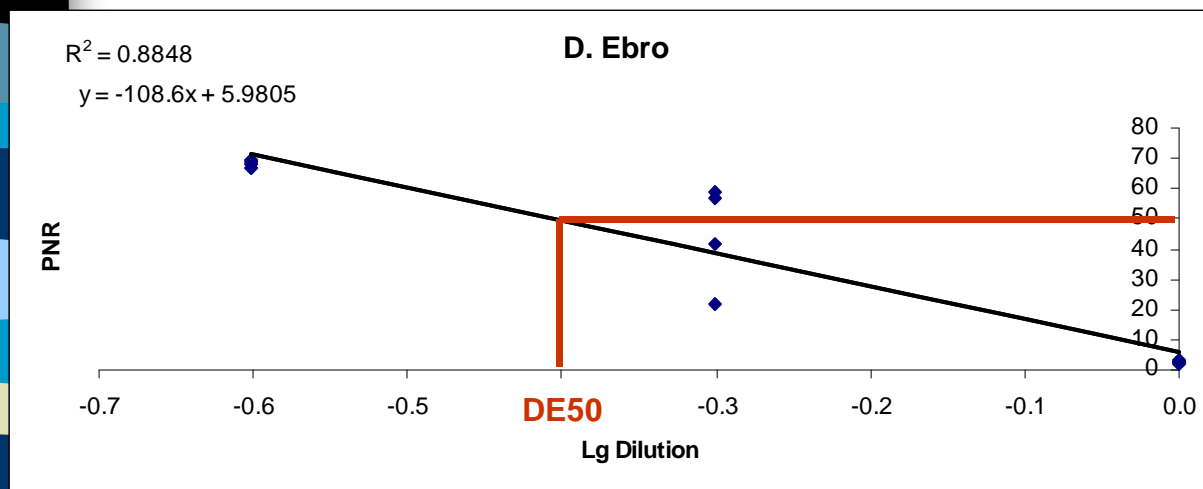
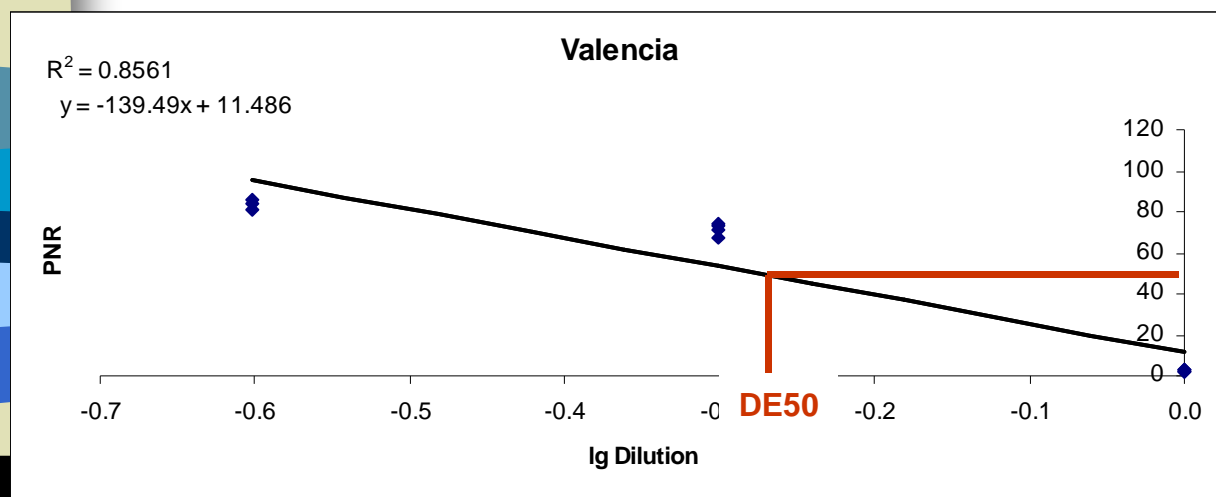


Pluteus = 300-450 μm

Durán and Beiras, 2010. *Environmental Toxicology and Chemistry*, 29(5): 1192:1198

Saco Álvarez et al., 2010. *Ecotoxicology and Environmental Safety*, 73: 491-499

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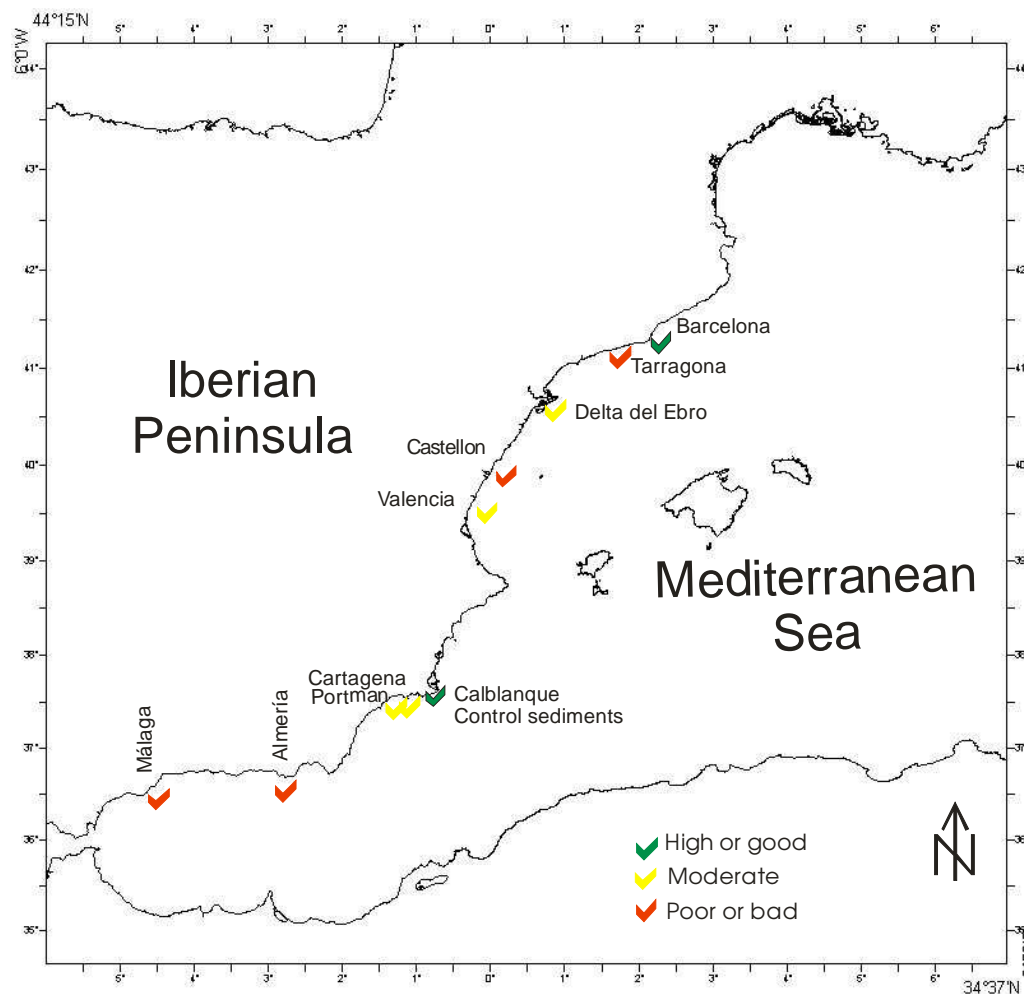


1. Linear regression
PNR vs Lg Dilution
2. DE50 = dilution
causing 50%
decrease
3. Obtention TU

TU (Toxic Units)

$$\text{TU} = 100 / \text{DE50}$$

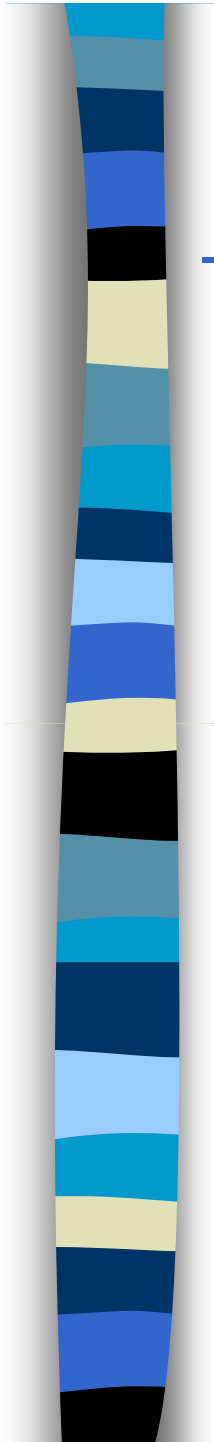
Sediment Quality Status	SET results		
	TU	PNR	"traffic-light" colour code
high or good	<0.27	>0.694	green
moderate	0.27 to 0.86	0.508-0.694	yellow
poor or bad	>0.86	<0.508	red



Preliminary Results of SET test in Biomonitoring program along Iberian inner shelf

Martínez-Gómez et al., 2010. IEO. Centro oceanográfico de Murcia . *Unpublished results. Not to be cited without prior request to the author.*

Establishment of ACs for Biological responses in BMIS program



FUTURE DEVELOPMENT

- To analyse more recommended MPIs-related in fish from fixed areas: Histochemical techniques and diseases.

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- To incorporate other recommended MPIS-related with biodiversity in BMIS: benthic index in sediments
- Different levels of biological organizations: To Integrate BMIS programme with fisheries monitoring surveys....> Crossing data

THANK YOU

MERCIE

